

Effect of S-mustard on Stress Response

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ABSTRACT

The cellular stress response is an universal defence mechanism, by which so-called stress proteins are induced and expressed on the expense of the constitutive protein expression, following cellular exposure to stress effectors of various kind including heat shock. The heat shock response in mononuclear human cells was investigated with respect to any effect of sulfur mustard on this response. The results showed a strong, inhibitory effect on the heat shock response in sulfur mustard (0,1 mM) poisoned cells, which included strong inhibition of both stress protein and constitutive protein synthesis. The results further showed that this effect was strongly dependent on the order of mustard poisoning and heat shock, since heat shocked cells exposed to sulfur mustard displayed a normal heat shock response. The results indicate a special mechanistic coupling between stress response and sulfur mustard poisoning, which might have both biochemical (transcriptional/translational) as well as physiological consequences.

94-07955



20030310205

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INTRODUCTION

In spite of several research efforts the mechanism of contact injury caused by sulfur mustard is still unrevealed, so that a medical defence has to concentrate on symptomatic treatment of the injuries. This means that the search for new knowledge on toxic effects versus cell survival has to be continued. Sulfur mustard is an antiproliferative agent due to its alkylating effect, which leads to inhibition of DNA replication and/or to DNA repair with subsequent NAD^+ and energy depletion [1]. Besides effect on DNA, inhibitory effect by the sulfur mustard on constitutive protein synthesis has been reported, which was suggested as a potential cause of contact injury [2].

An interesting aspect concerning cell survival in general is the cellular stress response found in most cells studied. During this response, a limited number of proteins for cellular defence are rapidly induced and expressed whereas the constitutive protein expression is reduced [reviewed in ref. 3-8]. These so-called stress proteins (also named heat shock proteins), which include the major Hsp70 and Hsp90, are induced following exposure of cells to either of a wide variety of stress effectors among which heat shock is the classical one. Due to the universal importance of the cellular stress response, the present investigation was undertaken to reveal whether sulfur mustard might affect this response. The experiments were performed on human mononuclear cells with heat or cadmium ions as stress effector. Both the stress protein and constitutive protein expression were found to be inhibited by sulfur mustard if the incubation with mustard preceded the incubation with the stress effector, or was performed concomitantly. No inhibition was observed if the mustard was added subsequently to the incubation with stress effector.

METHODS

Mononuclear cells were isolated from human blood or leucocyte concentrates (buffy coat samples) with Lymphoprep (Nycomed) as described by Bøyum [9,10]. The cells were pelleted and resuspended in methionine free medium (GIBCO BRL) before the incubations.

Each experiment consisted of successive incubations, using stress effector and sulfur mustard either concomitantly (Fig 1) or in various order within each series of experiments to be compared (Fig 2-3). The incubation with [^{35}S] methionine for metabolic labeling of the proteins, was performed at the end of each experiment and at identical time within each series of

experiments to be compared. All incubations were performed in incubator at 37°, except for the heat shock incubation at 43° in water bath.

After incubation the cells were pelleted and resuspended in pure water for lysis.

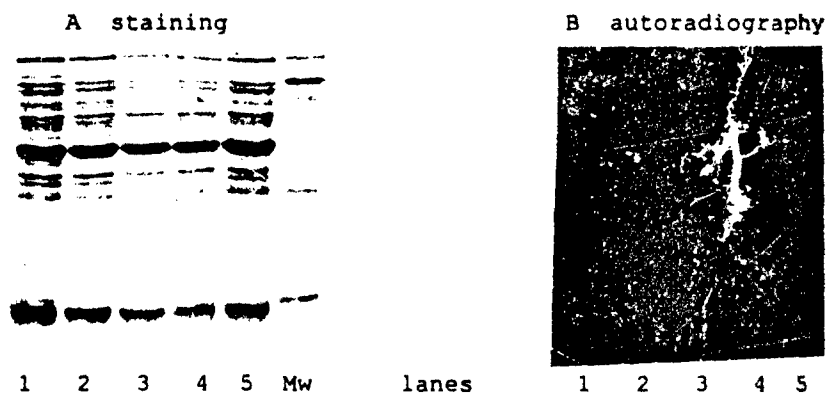
The proteins of the cell extracts were separated by SDS-polyacrylamide gel electrophoresis, according to the method by Laemmli [11], with 3% stacking gel and 12% separating gel.

After electrophoresis, the gels were stained with Coomassie brilliant blue G to detect the total protein content in the extracts, dried, and subjected to autoradiography to detect the protein expression during the incubation with radioactive methionine.

RESULTS

Fig 1 shows the results after gel staining (A) and autoradiography (B) for a series of experiments in which cells were heat shocked and concomitantly exposed to various concentrations of sulfur mustard.

FIG. 1



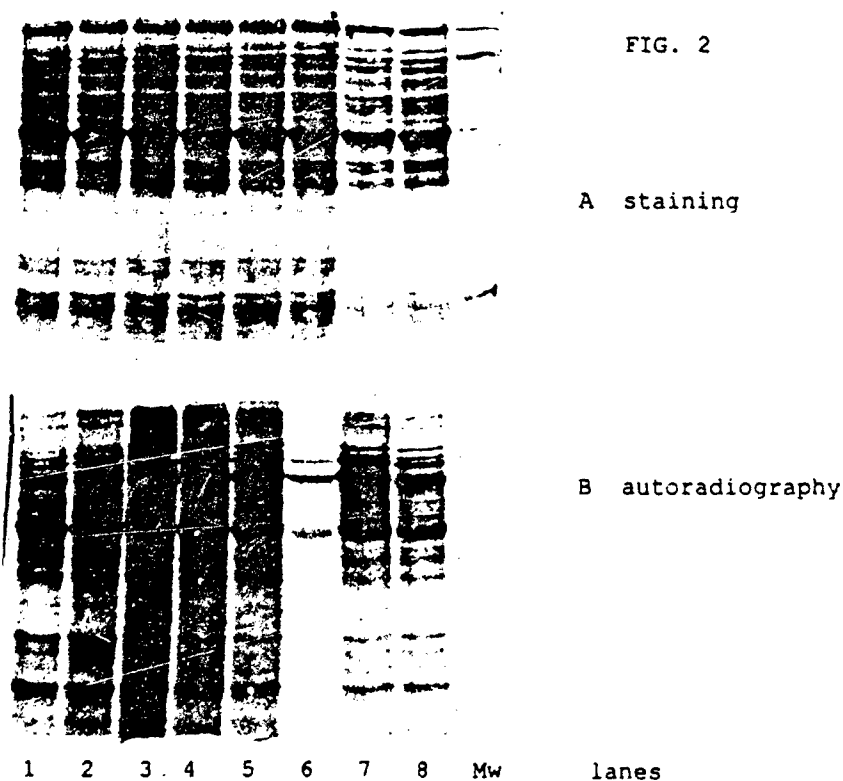
The total protein content seemed to be similar in the various extracts (Fig 1A, lane 1-5), which were from cells incubated without (lane 1) and with (lane 2) solvent addition (1% DMSO), or with 0,01mM (lane 3), 0,1mM (lane 4) or 0,5 mM (lane 5) sulfur mustard.

The autoradiogram (Fig 1B) showed almost total inhibition of the protein expression in cells added 0,1 or 0,5 mM sulfur mustard (lane 4 and 5), which contrasted to the expression of constitutive proteins and stress proteins Mw 70.000, Mw 90.000 (Hsp70 and Hsp90) in the other cells (lane 1-3).

The two stress proteins detected by autoradiography (Fig 1B, lane 1-3), could not be seen by the method of gel staining (Fig 1A, lane 1-3).

Fig 2 shows the results after gel staining (A) and autoradiography (B) for experiments which included cells incubated with sulfur mustard either before or after the heat shock.

The total protein content seemed to be similar in the various cell extracts (Fig 2A, lane 1-8).



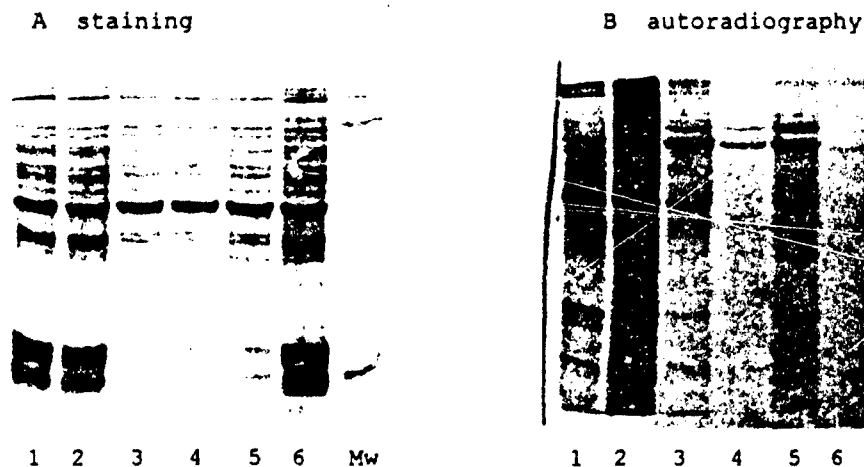
The autoradiography of the same gel (Fig 2B) showed strong inhibition of the protein expression in cells exposed to 0,1mM sulfur mustard and subsequently heat shocked (lane 6) compared to cells exposed and shocked in the invers order (lane 8). The labeling in the latter cells (Fig 2B, lane 8) showed similar intensity as in cells exposed to low concentration of sulfur mustard (0,01mM) either before (lane 5) or after (lane 7) heat shock.

The labeling in these cells (Fig 2B, lane 5,7,8) was also similar to the labeling in various control cells, which were either unshocked and added sulfur mustard (lane 1,2) or heat shocked and added solvent (lane 4), but it was lower than in unshocked cells added solvent (Fig 2B, lane 3).

Similar results as in Fig 2, were also obtained in parallel experiments performed with CdSO₄ incubation instead of heat shock as stress effector (results not shown).

Fig 3 shows the results from experiments with cells exposed to sulfur mustard (0,1mM) either before heat shock (lane 6) after heat shock (lane 5) or between two heat shocks (lane 4). The various controls included unshocked cells added sulfur mustard (lane 1) or solvent (1% isopropanol) (lane 2), and heat shocked cells added solvent (lane 3).

FIG. 3



The total protein content by gel staining differed marginally between the various cell extracts (Fig 3A), whereas the protein expression found by autoradiography differed strongly (Fig 3B). In any case if the cells were heat shocked subsequently to mustard exposure, i.e. whether or not a preceding heat shock was given, almost no expression of proteins could be observed (Fig 3B, lane 4 and 6) except for a slightly more intense labeling of the stress proteins in cells which were heat shocked twice (lane 4).

In cells heat shocked once, before the mustard exposure (Fig 3B, lane 5), all proteins were labeled to a similar extent as in heat shocked cells not exposed to mustard (lane 3).

DISCUSSION

In the present investigation a new effect of sulfur mustard in cells has been discovered.

The results show that after moderate exposure to sulfur mustard, mononuclear cells have lost their capacity to answer an ordinary stress effector through the cellular stress response (Fig 1B, lane 4,5, Fig 2B, lane 6, Fig 3B, lane 4,6). The results are interesting because the cellular stress response is universal, i.e. present in all cells investigated, and because stress effectors may be of several kinds including some cytokines and arachidonic acid metabolites [3-8] which may be relevant for the contact injury caused by sulfur mustard. The present effect might therefore have both biochemical as well as physiological consequences.

The inhibitory effect was dose dependent (Fig 1B, lane 3-5, Fig 2B, lane 5,6), and it was fully developed at a concentration of sulfur mustard (0,1 mM) which is fairly below the suggested vesicating dose (\approx 0,25 mM [2]).

Also, the effect was strongly dependent on the order of the exposures to sulfur mustard and stress effector (Fig 2B, lane 6 compared to lane 8, Fig 3B, lane 6 compared to lane 5), and it could not be counteracted by another preceding stress exposure (Fig 3B, lane 4 compared to lane 6). These results points to both transcriptional and translational sites of effect, which might be of importance within futural sulfur mustard research as well as stress response research. However, the interpretation of the results should not be confused with effects on transcription and /or translation in general. Stressed cells did express both the stress proteins and constitutive proteins whether or not they were exposed to sulfur mustard subsequent to the stress effector (Fig 2B, lane 4 versus lane 8, Fig 3B, lane 3 versus

lane 5). Therefore, there has to be a special mechanistic coupling between the stress response and effect of sulfur mustard.

It is worth to remark that the observed effect of sulfur mustard on the protein expression during the stress response should not be an artefact due to any leakage of proteins from the cells, since the total protein content did not seem to differ between the extracts (Fig 1A, Fig 2A, Fig 3A).

CONCLUSIONS

1. A new effect of sulfur mustard in cells is discovered
2. Sulfur mustard poisoned cells have lost their capacity to perform the cellular stress response
3. The questions to be answered are:
 - A. to which extent does the new effect influence the contact injury caused by sulfur mustard
 - B. what is the mechanistic connection between the cellular stress response and sulfur mustard poisoning

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